Effects of chronic and repeated corticosterone administration in rearing chickens on physiology, the onset of lay and egg production of hens

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ABSTRACT

A corticosterone model was used to study the effects of chronic and repeated stress during the rearing phase on physiology, the onset of lay and performance of laying hens in the subsequent laying period. Two hundred and seventy Hy-line brown layer pullets were reared in environmentally controlled battery cages. At 7, 11, and 15 weeks of age birds were exposed for 1 week to the following treatments in drinking water: corticosterone dissolved in ethanol, ethanol, or untreated water. One week following each treatment, and at 35 weeks of age endocrine, metabolic and haematological tests were conducted. Body weight was measured throughout the study, and egg production was recorded daily throughout the laying period.

Plasma corticosterone levels and heterophil to lymphocyte (H/L) ratio were increased after each corticosterone delivery, showing the effectiveness of the treatment. When corticosterone delivery was interrupted, plasma corticosterone and H/L ratio were significantly reduced. Exposing birds to repeated and long-term corticosterone treatment significantly affected BW ($P<0.01$), and relative organ weights ($P<0.01$). Corticosterone delivery also resulted in increased blood levels of glucose (GLU), cholesterol (CHOL), and triglyceride (TRG). Administration of corticosterone during the rearing phase delayed the onset of lay and decreased egg production at 35 weeks of age. These results demonstrate that oral corticosterone treatment affects hen physiology, reduces performance, and may model the effects of production stressors.

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1. Introduction

For many years, researchers have investigated the effects of a variety of environmental factors on behavioral, physiological and performance responses of birds. From these studies it has been shown that conditions such as climatic [13,15,27,32], nutritional [11,14] social [2,15], and biological [15,27,29], as well as experimentally elevating circulating corticosterone [3,4,9,10,17,20,29,33] induce in poultry a state of stress response associated with an increased plasma corticosterone concentration, and a number of modifications to metabolic [4,12,24,29], physiological [17,20,21,29,33] and immunological functions [9,11,13,14,26,28,29,32]. Studies on the stress physiology of poultry have emphasized that corticosterone produced during acute or chronic stress is one of the final hormones of the hypothalamic–pituitary–adrenal (HPA) axis cascade [1,7], and plays a multifunctional role in the chicken's body through the alteration of neuro-endocrine and immune components [2,25].

However, the effect of a repeated and long-term (chronic) corticosterone administration to chickens during the rearing phase on physiological and performance status in the subsequent laying period has not been reported. This is of particular concern for laying-type birds reared under commercial conditions. Such birds are introduced to various stress such as repeated restraint (i.e., weighing, vaccination, moving to the layer shed etc.), and a variety of environmental changes. These stressors induce physiological changes that perturb endocrine, immune and metabolic homeostatic and allostatic mechanisms, and may impact on their growth and development during rearing [4,5,12]. Such mechanisms can also affect the physiology of hens, particularly the onset of lay and egg production in the subsequent laying phase [6,35].

In the present study we investigate the effects of repeated oral corticosterone administration in birds during the rearing phase on physiological responses and performance of growing birds and laying hens until the peak production (i.e. 35 weeks of age). The experimental rationale was to assess the effect of corticosterone by using multiple measures of stress including hen physiology and performance.

2. Materials and methods

2.1. Experimental design

Experiments were conducted with 270 Hy-line brown layer pullets. Four-week old birds were tagged and placed in stainless steel batteries, each holding six birds at a density of 5 birds per m^2 in an environmentally controlled house. Birds were allowed to adapt to

the experimental conditions for 3 weeks. They were randomly assigned to 3 treatment groups, each of which comprised 15 replicate cages. Birds were moved to laying cages in the same room when they were 16 weeks of age (1 week after the third treatment with corticosterone). The light regimen was adjusted according to the breeder's recommendation and was appropriate for the rearing and laying period. The temperature was kept between 22 ± 2 °C during the entire experimental period. An appropriate diet (grower-, finisher- and layer-diet) and water was provided *ad libitum* during the whole experimental period.

Our previous experiments with dietary corticosterone have shown that administration of corticosterone in drinking water increased circulating corticosterone above the baseline and induced effects similar to responses to stressors [28,29]. Corticosterone (Sigma Aldrich Inc., St Louis MO, USA) was dissolved in alcohol (100%) and diluted in the drinking water to achieve a final concentration of 20 mg/L. At 7, 11, and 15 weeks of age birds were exposed for 1 week (to mimic the effect of chronic exposure to stress) to the following treatments in drinking water: corticosterone dissolved in alcohol (CORT), alcohol (Ethanol), or untreated water (Control). To facilitate corticosterone treatment, water was provided through a controlled bottle-pipe system. All procedures conducted in this study were in accordance with the "Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th ed. (2004) and Model Codes of Practice for the Welfare of Animals: Domestic Poultry, 4th ed. (2002) and were approved by the Animal Ethics Committee of the University of Queensland, Australia.

2.2. Measurements

Every day of the experimental period, all birds were checked for their health and welfare. Blood samples were taken from 15 randomly selected birds in each treatment (1 per cage) before the treatments started (day 0 or at 7 weeks of age), 7 days after each treatment (at 8, 12, and 16 weeks of age), and at 35 weeks of age. Whole blood was collected into vaccutainers containing lithium heparin (BD Diagnostics, UK) and was used to measure haematological parameters in an automated analyser (CELL-DYN® System 3700CS, Abbott Park, IL 60064). Results obtained from the haematology analyser were used for the absolute and relative blood cell counts and to calculate heterophil to lymphocyte (H/L) ratios. The blood samples were then centrifuged at 1500 ×g for 10 min and the plasma was divided into two aliquots and stored at −20 °C for corticosterone and plasma metabolites measurements.

At 35 weeks of age, 6 birds per treatment were randomly selected and euthanized to collect organs (spleens, livers and reproductive tracts). Body weight (BW) and egg production i.e., hen day production (HDP) were measured throughout the study until 35 weeks of age.

2.3. Plasma analysis

Corticosterone was measured by enzyme-immunoassay using a commercial kit, OCTEIA CORT HS (Immunodiagnostic Systems Ltd., Bolton, UK). All samples were run in duplicate and kit calibrators and controls were included in each analysis. Absorbance was measured at 450 nm, with a reference wavelength of 650 nm, in an ELISA microplate reader (MRX® II Dynex Technologies, USA). Blood concentrations for the plasma metabolites glucose (GLU), cholesterol (CHOL) and triglycerides (TRG) were determined using commercial kits and a chemistry system (VetTest chemistry analyser, IDEXX Laboratories, Inc. USA). The inter-assay coefficients of variation for corticosterone, GLU, CHOL and TRG were 9.2%, 8.4%, 8.1% and 6.9%, respectively. The intra-assay coefficient for corticosterone was 3.9%.

2.4. Statistical analysis

Data were analysed using a two-way repeated measures ANOVA for the effects of treatment and time on each variable (corticosterone,

Table 1
Effects of corticosterone administration on plasma corticosterone concentration, H/L ratio and plasma metabolites of laying hens at 7, 8, 12, 16 and 35 weeks of age[#].

Parameters and treatments	Age*				
	7 weeks	8 weeks	12 weeks	16 weeks	35 weeks
CORT (ng/ml)					
Control	4.14 ± 0.15^{x}	3.33 ± 0.21^{y}	3.55 ± 0.17^{y}	4.72 ± 0.15^{y}	4.30 ± 0.15^{x}
Ethanol	4.24 ± 0.17^{x}	4.65 ± 0.16^{y}	3.69 ± 0.20^{y}	4.30 ± 0.25^{y}	4.78 ± 0.20^{x}
CORT	$4.30 \pm 0.12^{c,x}$	$13.5 \pm 0.12^{a,x}$	$12.7 \pm 0.45^{a,x}$	$11.2 \pm 0.18^{ab,x}$	$5.33 \pm 0.15^{c,x}$
H/L ratio					
Control	$0.22 \pm 0.03^{b,x}$	$0.22 \pm 0.02^{b,y}$	$0.25 \pm 0.04^{ab,y}$	$0.20 \pm 0.05^{b,y}$	$0.30 \pm 0.07^{a,x}$
Ethanol	$0.24 \pm 0.01^{b,x}$	$0.25 \pm 0.02^{b,y}$	$0.24 \pm 0.01^{b,y}$	$0.24 \pm 0.04^{b,y}$	$0.32 \pm 0.04^{ab,x}$
CORT	$0.23 \pm 0.02^{c,x}$	$0.88 \pm 0.07^{ab,x}$	$0.84 \pm 0.04^{ab,x}$	$1.08 \pm 0.07^{a,x}$	$0.34 \pm 0.03^{bc,x}$
GLU (mg/dl)					
Control	258 ± 3^{x}	257 ± 9.0^{y}	256 ± 3.6^{y}	250 ± 5.4^{y}	274 ± 7.2^{x}
Ethanol	259 ± 3^{x}	252 ± 3.6^{y}	247 ± 1.8^{y}	243 ± 3.6^{y}	259 ± 1.8^{x}
CORT	$260 \pm 2^{c,x}$	$448 \pm 12.6^{a,x}$	$369 \pm 7.2^{ab,x}$	$328 \pm 5.4^{b,x}$	$281 \pm 3.6^{c,x}$
CHOL (mg/dl)					
Control	86 ± 1^{x}	88 ± 5^{y}	90 ± 3^{y}	91 ± 2^{y}	129 ± 4^{x}
Ethanol	88 ± 4^{x}	89 ± 3^{y}	90 ± 4^{y}	88 ± 2^{y}	134 ± 2^{x}
CORT	$89 \pm 1^{c,x}$	$211 \pm 7^{a,x}$	$195 \pm 7^{ab,x}$	$129 \pm 4^{b,x}$	$130 \pm 4^{b,x}$
TRG (mg/dl)					
Control	58 ± 0.6^{x}	62 ± 4^{y}	59 ± 3^{y}	59 ± 4^{y}	1118 ± 2^{x}
Ethanol	57 ± 0.8^{x}	58 ± 6^{y}	60 ± 5^{y}	58 ± 6^{y}	1165 ± 4^{x}
CORT	$53 \pm 1.0^{c,x}$	$144 \pm 3^{b,x}$	$166 \pm 5^{b,x}$	$123 \pm 7^{b,x}$	$1168 \pm 5^{a,x}$

[#]Values are mean ± SEM, $n = 15$.
CORT = corticosterone; H/L = heterophil to lymphocyte; GLU = glucose; CHOL = cholesterol; TRG = triglyceride.
Control = untreated water treated; Ethanol = alcohol; CORT = corticosterone dissolved in alcohol.
*Data presented are at 7 (baseline), 8, 12, and 16 weeks (wks) of age (or 1 week after each corticosterone treatment), and at 35 weeks of age.
[a–c]Means with different superscripts within the same treatment differ significantly ([a–b]$P<0.05$; [a–c]$P<0.01$).
[x–y]Means with different superscript within the same time period differ significantly ([x–y]$P<0.05$).

H/L ratios, GLU, CHOL, TRG, BW and HDP) at 7, 8, 12, 16 and 35 weeks of age. Organ weights were compared between different treatment groups using one-way ANOVA. Treatment means at the different times were analyzed using the multiple range Duncan test for mean separation, with significance set at $P<0.05$. Correlations between different significant measures were determined using Pearson's correlation coefficient. All analyses were performed using the GLM procedure of SAS [23].

3. Results

3.1. Corticosterone, H/L ratio and plasma metabolites

The results for plasma corticosterone concentration, H/L ratio, and plasma metabolites (GLU, CHOL, and TRG) at 7, 8, 12, 16, and at 35 weeks of age are presented in Table 1. At 7 weeks of age (before the treatment started) there were no significant variations between and within birds and groups (Control, Ethanol and CORT). Plasma corticosterone concentrations and H/L ratios were increased immediately (1 h post-first treatment with corticosterone; data not presented here), and continued to remain elevated 1 week (i.e. at 8 weeks of age) post-repeated treatment with corticosterone [CORT: $F_{(3,32)} = 11.25$, $P<0.01$; H/L ratio $F_{(3,32)} = 8.54$, $P<0.01$] (Table 1), showing the effectiveness of the treatment. However, repeated treatment with corticosterone at 11 and 15 weeks of age did not supplementarily increase plasma corticosterone concentration at 12 and 16 weeks of age [$F_{(3,50)} = 2.206$, $P = 0.1$ and $F_{(3,50)} = 0.77$, $P = 0.51$; respectively] compared to previous measurements (at 8 and 12 weeks of age, respectively). When corticosterone delivery was interrupted plasma corticosterone levels and H/L ratios were reduced (3 days later or on day 10) and did not differ significantly compared to basal levels and control and ethanol-treated chickens (data not shown). At 35 weeks of age, plasma corticosterone concentration was unchanged [$F_{(1,16)} = 0.013, P = 0.91$] (Table 1), whereas H/L ratio was numerically increased [$F_{(1,16)} = 5.28$, $P<0.004$] in all groups when compared to basal levels (or 7 weeks of age), showing a significant effect of age. There was a significant ($P<0.001$) positive correlation (Pearson's $r = 0.897$) between plasma corticosterone concentration and H/L ratio.

Corticosterone treatment resulted in increased blood levels of GLU, CHOL, and TRG. Dietary corticosterone caused a marked hyperglycaemia [$F_{(2,27)} = 39.16$, $P<0.01$] 1 week post first treatment (Table 1). However, GLU levels were significantly lower 1 week after the second treatment with corticosterone (at week 12 of age) compared to levels at 8 weeks of age, yet significantly higher [$F_{(2,23)} = 9.15$, $P<0.002$]

Table 2

Effects of corticosterone administration on BW and proportional weights of spleen, liver and reproductive organs of laying hens at 35 weeks of age*.

Parameter[1]	BW (g)	Spleen g/100 g BW	Liver g/100 g BW	Oviduct g/100 g BW	Ovary g/100 g BW
Control	2051 ± 45^a	0.11 ± 0.01^a	2.63 ± 0.14^a	2.49 ± 0.08^a	2.68 ± 0.11^a
Ethanol	2078 ± 28^a	0.11 ± 0.01^a	2.37 ± 0.11^a	2.51 ± 0.13^a	2.62 ± 0.09^a
CORT	1813 ± 39^b	0.07 ± 0.01^b	3.48 ± 0.18^b	1.65 ± 0.05^b	1.96 ± 0.08^b

*CORT administration was discontinued at 16 weeks of age.
[1]Values are mean $\pm$ SEM, $n = 6$.
Control = untreated water treated; Ethanol = alcohol; CORT = corticosterone dissolved in alcohol.
a–bMeans with different superscripts within the same column differ significantly (a–b$P<$ 0.05.

compared to control and ethanol treatment, and basal levels. The GLU concentration continued to be significantly elevated also after the third treatment with corticosterone, but was not significantly different [$F_{(1,5)} = 5.00$, $P>0.05$] at 35 weeks of age (or ca. 20 weeks after last treatment with corticosterone) compared to control and ethanol groups and basal levels. The concentration of CHOL in the plasma was increased after each treatment with dietary corticosterone, however, the effect decreased with time [from 211 ± 7 at 8 weeks of age to 129 ± 4 at 16 weeks of age, $F_{(2,27)} = 20.21$, $P<0.01$; to 130 ± 4 at 35 weeks of age, $F_{(2,27)} = 10.30$, $P<0.01$) but was not different between 16 and 35 weeks of age ($P>0.05$)]. At 35 weeks of age, there was a significant increase in plasma CHOL concentration of all (corticosterone and ethanol-treated and control) birds compared to basal levels. Plasma TRG levels peaked after the second treatment with corticosterone [166 ± 5; $F_{(3,15)} = 23.70$, $P<0.01$], declined after the third treatment, and were significantly upregulated in all birds (independent of treatments) at 35 weeks of age.

3.2. Body weight and organ weights

Exposing birds to repeated and chronic corticosterone administration significantly affected BW ($P<0.01$) and proportional organ weights ($P<0.01$) at 35 weeks of age (Fig. 1 and Table 2). At all measurement points, corticosterone-treated birds had decreased body weight compared to both control and ethanol-treated chickens (Fig. 1). The decrease in BW started after the first week of treatment with corticosterone (at week 8) and continued until 35 weeks of age (end of the experimental period) even though corticosterone treatment was discontinued at 16 weeks of age. Relative (proportional)
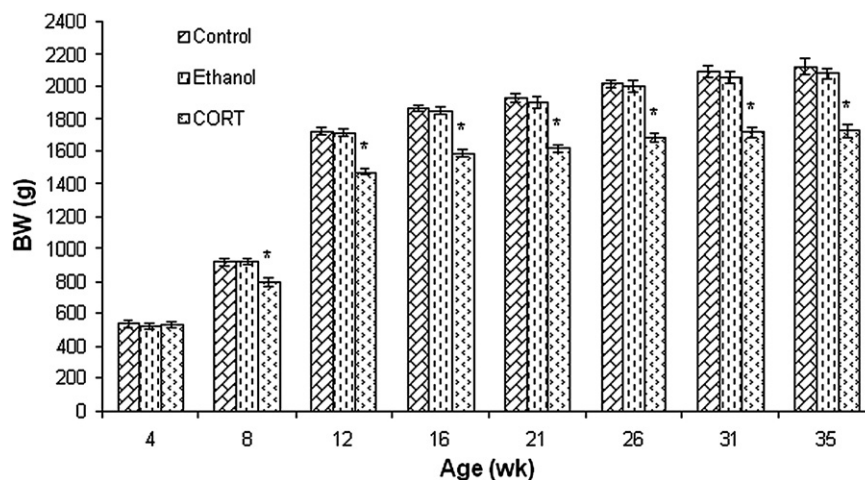

Fig. 1. Body weight from 4 to 35 weeks of age in treated and control chickens. Control = untreated water treated; Ethanol = alcohol; CORT = corticosterone dissolved in alcohol; Treatments with corticosterone were made at 7, 11 and 15 weeks of age. Values are mean $\pm$ SEM, $n = 90$; *indicates a significant difference from respective control groups at that time period ($P<0.05$).
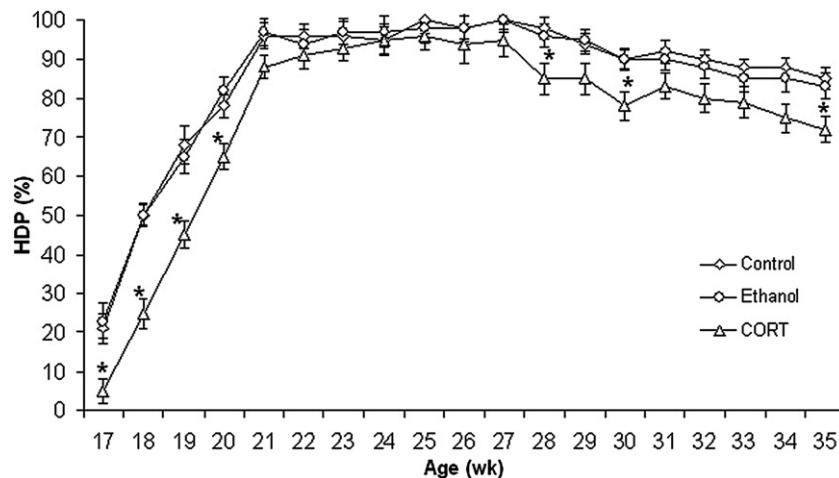
Fig. 2. Hen day production (HDP) (%) from 17 to 35 weeks of age in treated and control chickens. Control = untreated water treated; Ethanol = alcohol; CORT = corticosterone dissolved in alcohol. Values are mean ± SEM, $n = 90$; *indicates a significant difference from respective control groups at that time period ($P < 0.05$).

weights (g organ/kg BWx100) of spleen, liver, oviduct and ovary are summarised in Table 2. At 35 weeks of age the proportional weight of spleen was significantly lower in hens treated with corticosterone as compared to control and ethanol-treated birds (0.07 ± 0.01 vs. 0.11 ± 0.01), whereas liver weight was greater in corticosterone than in the control and ethanol treatments, even though corticosterone treatment was discontinued at 16 weeks of age. The decrease in body weight and relative spleen weight of corticosterone treated chickens were inversely proportional to the plasma corticosterone concentrations (Pearson's $r = -0.812$; $P < 0.001$). At 35 weeks of age ovary and oviduct weights were lower ($P < 0.05$) in corticosterone treated hens compared to control and ethanol treatments.

3.3. Onset of lay and egg production

Elevated corticosterone significantly delayed ($P < 0.05$) the onset of laying cycle and decreased ($P < 0.01$) egg production (Fig. 2). Corticosterone-treated laying chickens initiated laying an average of 8 days later, and reached the peak of laying 4 weeks later than control and ethanol-treated hens. Then, at 25 weeks of age egg production started to decline in birds treated with corticosterone and was significantly lower than both control and ethanol-treated hens ($P < 0.01$) at 35 weeks of age.

4. Discussion

The purpose of this study was to investigate the effects of chronic and repeated corticosterone administration in drinking water on physiological response, the onset of lay and performance of growing birds and laying hens. Overall, chronic (many times a day for 1 week) and repeated (3 times, or each 4 weeks until start of lay) exposure to corticosterone in drinking water elevated plasma corticosterone concentrations and H/L ratios to high physiological (stress-induced) levels and indicated that the method was effective and comparable to exposure to a chronic stressor. Additionally, it was shown that elevations of plasma corticosterone concentrations significantly alter metabolic processes and growth, and delay/reduce egg laying, which reflects the biological cost of the adaptation process. These observations are in accordance with previous studies conducted in broilers [12,20,21,33] and laying hens [4,18,29,34]. It should be pointed out that there is limited data on the effect of glucocorticoids (GCs) on laying hens. Mumma et al. [17] demonstrated that adrenocorticotropic hormone (ACTH) delivery stimulated BW gain in adult layer hens. This study indicates that the suppressive effects of exogenous corticosterone on BW can be persistent from rearing and throughout the laying period. Previous studies with other laying birds have shown that exogenous GCs inhibit the rate of body growth and/or can be passed on to the offspring of treated breeders [5].

Increases of both plasma corticosterone concentration and H/L ratio, are the most sensitive and established indicators of stress response in the chicken [10]. It should be noted that acute exposure to the stressor causes a short-lasting and rapid increase in stress hormones, with a tendency to returning to normal (baseline) once the stressor is removed or is diminished (i.e., corticosterone administration). But, if the presence of the same stressor is chronic and repeated, hormonal responses will be longer in duration and often associated with adaptation and attenuation of stress response [19], as demonstrated in this study. However, an understanding of plasma corticosterone and H/L ratio responses to chronic and repeated stressors separated by weeks requires an appreciation of the endocrine and physiological status after an initial exposure and after the last stressor exposure. It is vital that the HPA-axis response to the stressor is maintained during chronic stress. Adaptation of the HPA axis to stress relies on complex interplay between multiple body systems [8]. Continued elevation of blood corticosterone concentrations (long-lasting stress) will cause prolonged alterations in many of the body's systems such as immune, cardiovascular, gastrointestinal and reproductive systems [25].

The present study simultaneously measured multiple physiological (endocrine, immune, metabolic and reproductive) and performance parameters in the same corticosterone-treated and control birds from 7 to 35 weeks of age. Elevated plasma corticosterone concentrations consistently resulted in changes in most parameters measured (at 8, 12, and 16 weeks of age) and consequently affected laying performance of hens at the onset of lay until 35 weeks of age. Apparently, the alteration of the mechanisms that regulate metabolic processes participating in growth and/or egg production continued to persist despite a decline in plasma corticosterone levels. Corticosterone added to the drinking water caused a shift in resources in the birds from growth to the synthesis of lipids by activating gluconeogenesis and glucogenolysis, and inhibiting GLU intake into peripheral tissues; processes that led to the elevation of plasma GLU, increases in plasma concentrations of CHOL and TRG 1 week post-each treatment, and increase of liver weight (due to increase in fat content). Chronic stress has been shown to result in a negative energy balance [1]. However, it is difficult to describe the general hormonal and physiological changes underlying this shift in metabolism during chronic stress because results change across different model species (and their age) and different protocols (doses and routes of GC administration) and are not necessarily comparable. In chickens, plasma GLU concentrations have

been reported to increase [1,4,29] whereas TRG are reported to decrease [5] or increase [12,29]. The liver of corticosterone-treated birds weighed significantly more than those of the control birds while the ovary and oviduct were substantially regressed in the former. This increase in liver weight was in agreement with Pilo et al. [18] who studied the effects of corticosterone infusion on the lipogenic activity and ultrastructure of the liver of laying hens.

Exposure of chickens to corticosterone during the rearing phase caused reproductive failure manifested by a delay of first egg laid (for about 8 days) and reduction of egg production during entire laying period (in this experiment from 18 to 35 weeks of age). The reduction of reproductive performance associated with stress is a known phenomenon in domestic birds [1,35]. In this study only 5% of birds initiated laying at 17 weeks of age compared to 22–23% of birds in control and ethanol-treated groups. Many studies have also documented an association between elevated levels of corticosteroids and suppression of reproductive behaviour [16,30,31]. However, it is not clear if the suppression of egg production is indicative of involvement of corticosterone in metabolic changes associated with egg production, or if it reflects a response to the energetic stress of reproduction. Etches et al. [6] have shown that corticosterone may modulate the responsiveness of the hypothalamus to gonadotropic stimuli and demonstrated that exposure to corticosterone can alter the responsiveness of some ovarian tissues to gonadotropins.

The results of the present study clearly support the hypothesis that chronically elevated concentrations of plasma corticosterone inhibit not only BW but also relative immune organ weight (such as spleen). Involution of lymphoid organs i.e. spleen occurs in birds following ACTH and corticosterone [26], and has been explained by the depletion effect on lymphocytes from germinal cells [9].

It is clear that the neuro-endocrine and immune systems interact to maintain homeostasis when an organism is under severe or chronic stress. These systems utilize neurotransmitters, hormones, and cytokines for communication and regulation of biological systems. Catecholamines, GCs and cytokines all respond at first to help the body adapt when stressors activate the HPA axis. Normally, a feedback system protects the organism by down-regulating these mediators [22], but continued elevation of stress mediators overwhelm the organism, resulting in biological consequences such as inhibition of immunity, growth and reproduction.

In this study, chronic and repeated exposure to corticosterone caused a sustained elevation in plasma corticosterone concentrations, indicative of prolonged stress. Based on the findings presented here, it would appear that exposing laying pullets during rearing to chronic and repeated corticosterone in drinking water might be an effective method to investigate the effects of chronic stress on hen physiology, laying behaviour and egg production during the later laying period. The model may be useful in assessing the effects of production stressors. Finally, it will be critical to determine how long corticosterone-induced consequences will last, and to understand the mechanisms that maintain these persistent changes and contribute to the endocrine and immune interactions.

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